Emergence and Characterization of Serotype G9 Rotavirus Strains from Africa

Nicola Page,[1,2] Mathew Esona,[2,4] George Armah,[2,5] James Nyangao,[6] Jason Mwenda,[7] Theresa Sebunya,[8] Gorav Basu,[8] Naidu Pyndiah,[9] Natasha Potgieter,[3] Annelise Geyer,[2] and A. Duncan Steele[2,10]

[1]Viral Gastroenteritis Unit, National Institute for Communicable Diseases, Sandringham, [2]Medical Research Council Diarrhoeal Pathogens Research Unit, Department of Virology, University of Limpopo Medunsa Campus, Pretoria, and [3]Department of Microbiology, University of Venda for Science and Technology, Thohoyandou, South Africa; [4]Gastroenteritis and Respiratory Viruses Laboratory Branch, Centers for Disease Control and Prevention, Atlanta, Georgia; [5]Noguchi Memorial Institute for Medical Research, University of Ghana, Legon, Ghana; [6]Kenya Medical Research Institute and [7]Institute of Primate Research, Nairobi, Kenya; [8]Department of Biological Sciences, University of Botswana, Gaborone, Botswana; [9]HIV/AIDS/STI Virology Laboratory, Central Health Laboratory, Cando, Mauritius; and [10]Department of Immunizations, Vaccines and Biologicals, World Health Organization, Geneva, Switzerland

Serotype G9 strains have been detected sporadically and in localized outbreaks in various African countries, including South Africa, Botswana, Malawi, Kenya, Cameroon, Nigeria, Ghana, Guinea-Bissau, Libya, and Mauritius. Serotype G9 strains were analyzed to investigate genogroup characteristics, including subgroup specificity, electropherotype, and P and G genotypes. In addition, the antigenic composition of the South African G9 strains was assessed. African G9 strains were associated with both DS-1–like characteristics and Wa-like characteristics, indicating the predisposition of G9 strains to frequently reassort. Despite these reassortment events, serotype G9 strains appear to maintain antigenic character in the outer capsid protein, as evident with the reaction of the South African G9 strains with the G9-specific monoclonal antibody F45:1. Phylogenetic analysis clustered African G9 strains geographically, regardless of genogroup characteristics, into 1 lineage (IIId). Two groups of G9 strains, originating in India and Japan, were identified in this lineage. Continuous surveillance of circulating rotavirus strains in Africa is vital to prepare for future vaccine implementation on a continent that clearly needs such preventative medicines.

Diarrhea remains a major contributor to the high rates of childhood mortality in Africa. It has been estimated that 1 of 6 children born in sub-Saharan Africa will die before 5 years of age and that one-fifth of these deaths will be from diarrhea [1]. Rotavirus is the single most important etiological agent of diarrhea, responsible for 20%–25% of all diarrheal cases [1, 2]. Improvements in sanitation and the availability of clean water have not decreased the rate of rotavirus diarrhea in developed countries, illustrating the need to develop vaccines as the first strategy of prevention [3]. The availability of a rotavirus vaccine may provide a new tool to address the problem of child survival in Africa [1].

The rotavirus genome consists of 11 segments of double-stranded RNA (dsRNA) that are easily separated by polyacrylamide gel electrophoresis. The relative migration of RNA segments are used to differentiate strains into long, short, supershort, and abnormal RNA profiles [4]. Surrounding the dsRNA are 3 protein layers: the core, inner capsid, and outer capsid. Epitopes on the inner capsid protein (VP6) determine group and subgroup specificity. Seven rotavirus groups (A–G) and 4 subgroups (SG; SGI, SGII, SGI+II, and SG non-I non-II) in group A have been identified. The outer capsid proteins, VP7 and VP4, are able to independently elicit neutralizing antibody responses and are classified ac-

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Reprints or correspondence: Dr Nicola Page, Private Bag x4, Sandringham, Gauteng 2131, South Africa (nicolap@nicd.ac.za).

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cording to G types and P types, respectively. There are 15 G types and 27 P types, of which 11 G and 11 P types have been found in humans [5–7].

Comparative analysis of the VP7 amino acid sequences from different human and animal serotypes identified regions that were divergent among serotypes, termed VR1–VR9 [8]. In addition, 6 antigenic regions (A, B, C, and F, corresponding to VR5, VR7, VR8, and VR9, respectively, and D and E) have also been identified on the basis of nucleotide sequence analysis of rotavirus variants resistant to neutralization by VP7-specific monoclonal antibodies [9, 10].

The typing of circulating rotavirus strains before 1990 included studies in Central African Republic [11], Gambia [12], Kenya [13], Nigeria [14], and South Africa [15]. In these African settings, serotypes G1, G2, G3, and G4 were predominant, and 22% of the rotavirus-positive samples were untypeable. Additional studies in Malawi [16], Kenya [17], Ghana [18], and Guinea-Bissau [19, 20] similarly identified serotypes G1–G4 as predominant. The improvement in molecular techniques, allowing G typing of cocirculating rotavirus strains, resulted in only 12% of the rotavirus-positive samples being untypeable.

At the World Health Organization–sponsored African Rotavirus Network workshops held during 1998–2004, genotypes were determined for rotavirus strains from Namibia, Botswana, Zimbabwe, Zambia, Tanzania, Kenya, Sudan, Nigeria, Cameroon, Ghana, Ivory Coast, Burkino Faso, Tunisia, and Mauritius. Similar to previous studies, serotypes G1–G4 were predominant, although G9 strains were detected in Ghana, Nigeria, Kenya [21], Botswana, and Cameroon. Further analysis of the untypeable strains from the African Rotavirus Network countries may result in the detection of unusual strains with altered primer binding sites or of rotavirus strains of animal origin.

Human G9 rotavirus strains were first detected in the United States in 1983 [22] and have been sporadically detected in samples from children with diarrhea in Japan [23, 24] and Thailand [25]. In India, serotype G9 strains were predominant during 1986–1988 and during 1992–1993 [26]. After 1995, G9 strains began to be detected more frequently and emerged as the fifth most important human serotype, rivaling the previously more common G1, G2, G3, and G4 serotypes in some settings [27]. Reports have also described G9 strains in North America [28], Latin America [29, 30, 31], Central America [32], China [33], and Africa [32]. In addition, serotype G9 strains have been isolated from animals, including pigs [34] and lambs [35].

Two rotavirus vaccines have been licensed and implemented in the expanded program of immunization schedules in many countries [36, 37]. The first, Rotarix (GlaxoSmithKline Biologicals) is a monovalent human rotavirus [36] bearing the most globally common human rotavirus serotype (G1P[8]). The second, RotaTeq (Merck), is a pentavalent bovine-human reassortant containing the surface proteins for serotypes G1, G2, G3, G4, and P[8] and retaining a bovine (WC3) genome backbone [37]. Although doubts originally surfaced with regard to the ability of these vaccine candidates to protect against the emerging G9 strains, subsequent trials have demonstrated that both vaccines are efficacious against G9 strains. The protection is probably attributable to the association of G9 strains with the P[8] genotype, which is present in both vaccine compositions [36, 37]. This article describes the characterization and molecular analysis of the genotype G9 strains from Africa and includes specimens from South Africa, Botswana, Kenya, Ghana, Cameroon, and Mauritius.

MATERIALS AND METHODS

Stool selection. South African rotavirus-positive stool samples ($n = 567$) were obtained from historical collections available at the Medical Research Council–Diarrhoeal Pathogens Research Unit (DPRU). Since 1983, diarrheal stool specimens have been collected from the Ga-Rankuwa Hospital in the North West Province as part of ongoing surveillance. These specimens have been analyzed in part and described elsewhere [15, 38–40]. In addition, diarrheal stool specimens were collected from pathology laboratories and hospitals in South Africa (A.D.S., unpublished data). Fecal suspensions and raw stool samples from these collections were stored at −20°C.

From March 1998 through April 1999, 60 rotavirus-positive stool specimens were collected from children aged <5 years from the Vhembe region of the Limpopo Province, South Africa (N. Potgeiter, unpublished data). From July 1997 through February 2000, 894 rotavirus-positive stool samples were collected from 3 health centers in the Upper East region of Ghana [41]. From July 1999 through May 2000, 124 rotavirus-positive stool samples were collected from children with diarrhea attending outpatient clinics in the Southwest and Western Provinces of Cameroon [42]. As part of an ongoing rotavirus surveillance project in Kenya during 1998–2002, 650 rotavirus-positive stool specimens were collected from clinics and hospitals in Nairobi and neighboring areas of Nanyuki, Kitui, Narok, and Nakuru (J. Nyangao, unpublished data) [43]. From February 1999 through April 2000, 248 stool specimens were collected from young children in clinics and hospitals in Nairobi and the surrounding area (J.M., unpublished data). During 1999, human rotavirus-positive stool samples ($n = 10$) were collected from young children in Gaborone, Botswana (T.S., unpublished data). During 2000, rotavirus-positive stool samples from Mauritius ($n = 40$) were sent to the DPRU for further analysis (N. Pyndiah, unpublished data).

Rotavirus detection. Various enzyme-linked immunosorbent assay (ELISA)–based kits have been used at the DPRU to determine the presence of rotavirus in stool samples from South African children. These include the Rotazyme II (Abbott Lab-

oratories) during 1982–1985, Rotavirus ELISA (Dakopatt) during 1985, Rotavirus EIA (International Diagnostics Laboratories) during 1986–1990, Rotascreen Latex Agglutination assay (Mercia Diagnostics) during 1988, Rotadan (Novamed) during 1991–1996, and IDEIA Rotavirus kit (Oxoid) since 1997. A 10% suspension of fecal material was made in distilled water and kept at 4°C until analysis. A 10% suspension of fecal material was made in distilled water, and stool samples from Ghana, Cameroon, and Kenya were screened using the IDEIA Rotavirus kit, according to the manufacturer's instructions. Specimens collected in Mauritius were screened using electron microscopy and polyacrylamide gel electrophoresis. Specimens collected in Botswana were screened using Rotavirus ELISA Kit (Trinity Biotech), and the results were confirmed using electron microscopy.

VP7 and VP4 genotyping of rotavirus strains. The rotavirus dsRNA was extracted and purified using the RNaid kit (BIO 101), according to the manufacturer's instructions. The samples were characterized according to VP7 and VP4 types with use of nested reverse-transcription polymerase chain reaction (RT-PCR) methods and primers described elsewhere [16, 26, 44, 45].

Subgroup (VP6) ELISA. The subgroup specificity of the serotype G9 strains was determined using an in-house monoclonal antibody ELISA, as described elsewhere [39]. The group-specific [46] and subgroup-specific monoclonal antibodies [47] were a kind donation from H. B. Greenberg (Stanford University, Stanford, CA).

Polyacrylamide gel electrophoresis. Rotavirus dsRNA was extracted and run as described elsewhere [38]. The gels were stained using the silver staining technique of Herring et al [48].

Cloning and sequencing of the VP7 and VP4 genes of serotype G9 rotavirus strains. First-round VP7 RT-PCR products of selected South African, Cameroonian, Ghanaian, and Mauritian G9 rotavirus strains were cloned into pGEM-T Easy Vector (Promega), according to the manufacturer's instructions. Plasmids were sequenced automatically using M13 forward and reverse primers by DNA Sequencing Laboratory, University of Cape Town, and Core DNA Sequencing Laboratory, University of Stellenbosch. First-round VP4 RT-PCR products of 4 South African G9 rotavirus strains (5001DB/97, 8253NN/98, 6818NN/99, and 6342LP/99) were also cloned and sequenced.

First-round VP7 RT-PCR products from selected G9 strains from Botswana and Kenya were cleaned using the QIAquick PCR Purification Kit (Qiagen) and were sequenced by Gene Care Molecular Genetics using forward and reverse primers (sBeg and End9), according to Gouvea et al [45]. Only partial sequences, lacking the 3′ and 5′ terminal ends of the VP7 genes, were obtained and submitted to GenBank. A phylogenetic tree was constructed using the TreeView Program [49], with genetic distances generated on DNAMan computer software.

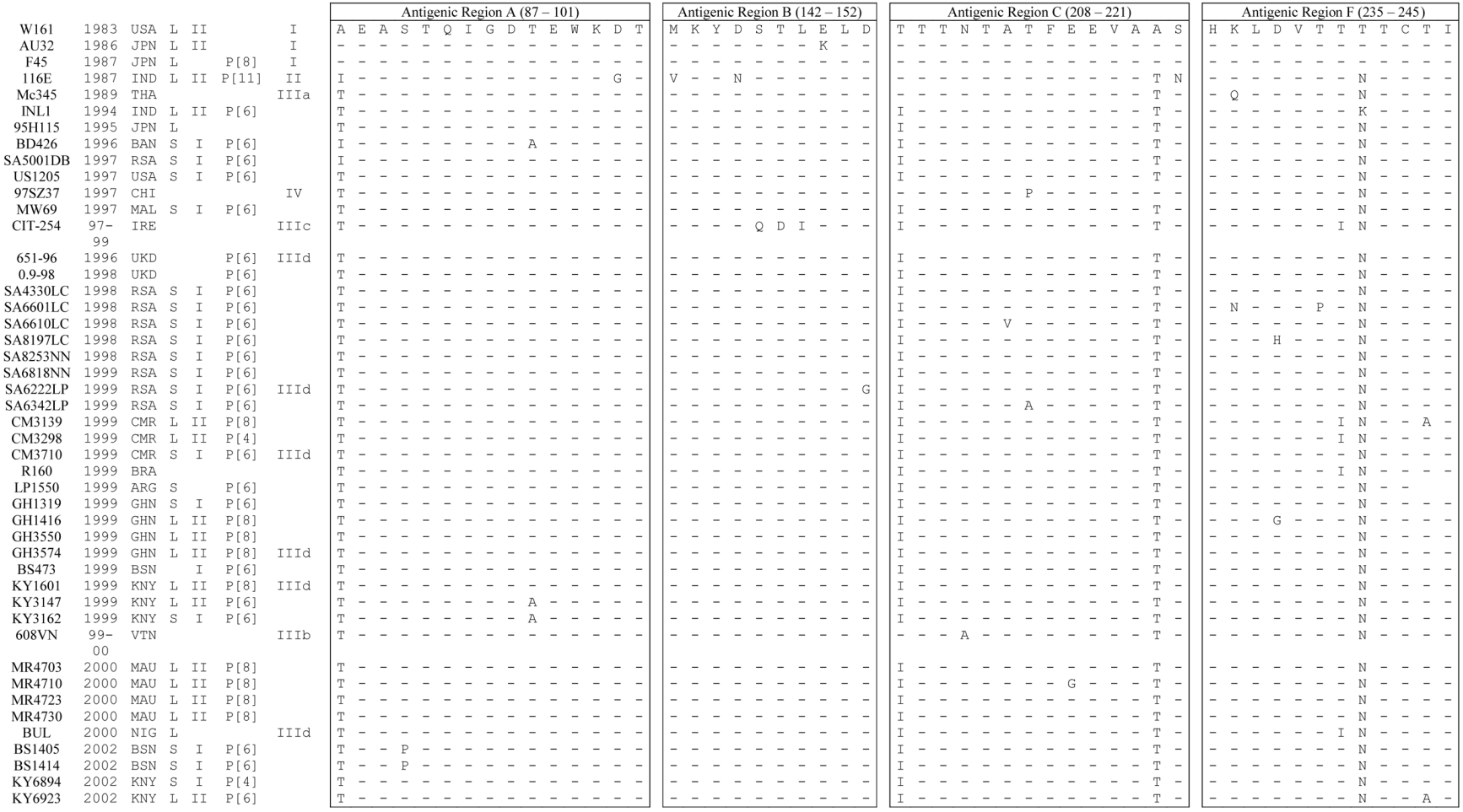
Nucleotide sequence accession numbers. The DNA sequences for the VP7 genes of the African G9 strains were submitted directly to GenBank and were assigned the following accession numbers: 5001DB/97, AF529864; 4330LC/98, AF529865; 6601LC/98, AF529866; 6610LC/98, AF529867; 8197LC/98, AF529868; 8253NN/98, AF529869; 6818NN/99, AF529870; 6222LP/99, AF529871 and 6342LP/99, AF529872; 3710CM/99, AY816184; 3139CM/99, AY816183; GH1319/99, AY211065; GH1416/99, AY211066; GH3550, AY21167; GH3574/99, AY211068; MR4703/00, AY262746; MR4710/00, AY262747; MR4723/00, AY262748, MR4730, AY262749; BS473/99, DQ822597; KY1601/99, DQ822600; KY3147/99, DQ822601; KY3162, DQ822602; BS1405/02, DQ822598; BS1414/02, DQ822599; KY6894/02, DQ822603; and KY6923/02, DQ822604. The DNA sequences for the VP8* genes of the South African G9 strains were submitted directly to GenBank and were assigned the following accession numbers: 5001DB/97, AF529873; 8253NN/98, AF529875; 6818NN/99, AF529876; and 6342LP/99, AF529874.

G (VP7) serotyping ELISA. VP7 serotyping was completed according to the method previously described by Taniguchi et al [50]. The following capture monoclonal antibodies were used: G1 [KU4], G2 [S2–2G10], G3 [YO-IE2], G4 [ST-2G7] [50] and G1 [5E8], G2 [IC10], G3 [159] [51], and G9 [F45:1] [52]. Mab60 [51] was included as a control to detect the presence of the intact double-shelled viral particles. Each plate contained negative controls, and the reactions were read spectrophotometrically at 450 nm.

RESULTS

Serotype G9 strains have been detected in various African countries, including South Africa, Botswana, Malawi, Kenya, Cameroon, Ghana, Libya, and Mauritius. The serotype G9 strains were sporadically isolated in South Africa, Botswana, Kenya, and Cameroon and appeared to be circulating at low levels in the population. Serotype G9 strains were, however, the predominant serotype in some settings, including the Upper East region of Ghana during 1999 and Mauritius during 2000.

South Africa genotype G9 strains. In South Africa, 7 G9P[6] strains with SGI specificity were isolated in the Gauteng and Limpopo Provinces during 1997–1999 (SA5001DB/97, SA4330LC/98, SA6601LC/98, SA6610LC/98, SA8197LC/98, SA6222LP/99, and SA6342LP/99) (Figure 1). In addition, serotype G9P[6] strains were predominant in the neonatal ward of the Dr George Mukhari Hospital, Pretoria, and 2 isolates were randomly selected for further analysis (SA8253NN/98 and SA6818NN/99). These strains were first detected in the latter part of 1998 and persisted in the ward during 1999, despite



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GH3574	1999	GHN	L	II	P[8]	IIId	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	I	-	-	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	N	-	-	-	-	-
BS473	1999	BSN		I	P[6]		T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	I	-	-	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	N	-	-	-	-	-
KY1601	1999	KNY	L	II	P[8]	IIId	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	I	-	-	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	N	-	-	-	-	-
KY3147	1999	KNY	L	II	P[6]		T	-	-	-	-	-	-	-	-	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	I	-	-	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	N	-	-	-	-	-
KY3162	1999	KNY	S	I	P[6]		T	-	-	-	-	-	-	-	-	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	I	-	-	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	N	-	-	-	-	-
608VN	99-00	VTN				IIIb	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	-	-	-	-	-	-	T	-	-	-	-	-	-	-	N	-	-	-	-	-
MR4703	2000	MAU	L	II	P[8]		T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	I	-	-	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	N	-	-	-	-	-
MR4710	2000	MAU	L	II	P[8]		T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	I	-	-	-	-	-	-	-	G	-	T	-	-	-	-	-	-	-	N	-	-	-	-	-
MR4723	2000	MAU	L	II	P[8]		T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	I	-	-	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	N	-	-	-	-	-
MR4730	2000	MAU	L	II	P[8]		T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	I	-	-	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	N	-	-	-	-	-
BUL	2000	NIG	L			IIId	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	I	-	-	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	N	-	-	-	-	-
BS1405	2002	BSN	S	I	P[6]		T	-	-	P	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	I	-	-	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	N	-	-	-	-	-
BS1414	2002	BSN	S	I	P[6]		T	-	-	P	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	I	-	-	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	N	-	-	-	-	-
KY6894	2002	KNY	S	I	P[4]		T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	I	-	-	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	N	-	-	-	-	-
KY6923	2002	KNY	L	II	P[6]		T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	I	-	-	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	N	-	-	A	-	-

Figure 1. Amino acid sequences of antigenic regions A (87–101), B (142–152), C (208–211), and F (235–245) of the VP7 gene of African G9 strains and selected international G9 strains. The strain name, year of isolation, country of origin, polyacrylamide gel electrophoresis (PAGE) profile (L or S), subgroup specificity (I or II), VP4 genotype (P[8], P[11], P[6], or P[4]), and G9 lineages (I, II, IIIa–d, or IV), as defined by Phan et al [61] are indicated where possible. ARG, Argentina; BAN, Bangladesh; BRA, Brazil; BSN, Botswana; CHI, China; CMR, Cameroon; GHN, Ghana; IND, India; IRE, Ireland; JPN, Japan; KNY, Kenya; MAL, Malawi; MAU, Mauritius; NIG, Nigeria; RSA, South Africa; THA, Thailand; UKD, United Kingdom; USA, United States; VTN, Vietnam.

the introduction of G1P[8] and G1P[6] strains in the ward during the rotavirus season in early 1999 [53].

The South African G9 strains were screened with the G9-specific monoclonal antibody F45:1, and 7 of the 9 selected G9 strains (SA5001DB/97, SA4330LC/98, SA6601LC/98, SA6610LC/98, SA8197LC/98, SA6818NN/99, and SA6222LP/99) could be detected using this monoclonal antibody. The failure of 2 samples (SA8253NN/98 and SA6342LP/99) to react with any of the monoclonal antibodies was more likely attributable to low viral titers, because these samples also failed to react with the group-specific Mab60.

Four of the South Africa G9 strains (5001DB/97, 8253NN/98, 6818NN/99, and 6342LP/99) were selected for cloning and sequencing of the VP8* subunit of the VP4 genes. The partial VP4 sequences obtained were submitted to GenBank for a BLASTn search on the National Centre for Biological Information Web site (http://blast.ncbi.nlm.gov/Blast.cgi. All VP4 sequences from the strains isolated were most homologous to the G9P[6] strain US1205 (AF079356), and the percentage of nucleotide homology ranged from 99.1% to 99.8% (data not shown).

African genotype G9 strains. From 1998 through 2001, 73 G9 strains were detected in Ghana, with the bulk of the SGII, long electropherotype G9P[8] strains ($n = 49$) being isolated in the Navrongo district during 1999 [41]. Four G9 strains, representing 3 RNA electrophoretic patterns, were selected for sequencing (GH1319/99, GH1416/99, GH3550/99, and GH3745/99) (Figure 1).

In Cameroon, 8 G9 strains (2 with SGI, P[4] or P[6] genotype, short RNA profiles and 6 with SGII, P[8], long RNA profiles) were detected. Three isolates bearing the respective genogroup characteristics were selected for sequencing (3139CM/99, 3298CM/99, and 3710/99) (Figure 1). The predominant strain in both provinces during that time was G1P[8] [42].

Serotype G9 strains were first detected in Nairobi, Kenya, during 1999. The first strain, KY3147/99, displayed a long electropherotype, SGII specificity, and P[6], and the second, KY3162/99, displayed a short electropherotype, SGI specificity, and P[6] (Figure 1). Another G9 strain (KY1601/99), with a long RNA profile, SGII specificity, and P[8] genotype (Figure 1), was collected in November 1999 from a child of unknown age in Kibera, the largest African slum located southwest of Nairobi city center (J.M., unpublished data).

The collection of stool specimens continued in Nairobi during 2002, and an additional 8 G9 strains were detected (J.N., unpublished data). Five strains possessed a long electropherotype, SGII specificity, and P[8]; 2 had long electropherotype, SGII specificity, and P[6], and a single strain showed a short electropherotype, SGI, and P[4] (data not shown). Two strains, KY6894/02 and KY6923, were selected for further analysis (Figure 1).

Despite the limited number of stool samples collected in Botswana in 1999, a single SGI G9P[6] strain was identified among the predominant G1 strains circulating (BS473/99) (Figure 1). Three years later, 4 SGI G9 strains were detected in stool samples from Botswana (G.B., unpublished data). Two of these strains (BS1405/02 and BS1414/02) were randomly selected for further analysis (Figure 1).

During 2001, rotavirus-positive stool samples were sent to the DPRU for further analysis (N. Pyndiah, unpublished data). The majority of the samples ($n = 40$) collected in 2000 were SGII, long electropherotype, G9P[8] strains ($n = 22$). Strains MR4703/00, MR4710/00, MR4723/00, and MR4730/00 were randomly selected for sequencing (Figure 1).

Analysis of VP7 antigenic regions and phylogeny. The amino acid sequences of antigenic regions A (amino acids 87–101), B (amino acids 142–152), C (amino acids 208–221), and F (amino acids 235–245) of African G9 strains and selected international G9 strains were aligned and compared. Analysis of the African G9 strains revealed point mutations at amino acid positions 87, 208, 220, and 242 (Figure 1). Additional point mutations in antigenic regions A and B were detected in strains from South Africa (SA6222LP/99), Kenya (KY3147/99 and KY3162/99), and Botswana (BS1405/02 and BS1414/02). In antigenic region C, point mutations were noted in SA6610LC/98, SA6342LP/99, and MR4710/00, and samples with SA6601LC/98, SA8197LC/98, CM3139/99, CM3298/99, GH1416/99, and KY6923/02 displayed point mutations in antigenic region D.

Phylogenetic analysis including African G9 strains and international strains from various countries was performed to determine the relationship between African G9 strains and those isolated on other continents (Figure 2). All the African G9 strains clustered in lineage IIId and tended to cluster geographically and not on the basis of genogroup characteristics.

DISCUSSION

The emergence of serotype G9 strains has prompted researchers in various countries to include reagents capable of detecting this once rare serotype. Serotype G9 strains (MW69) were first identified in Africa in Malawi from July 1997 through January 1998 [16]. Part of this study attempted to identify serotype G9 strains in South Africa over the past 2 decades, and improved typing protocols have allowed additional analysis to be performed on historical stool collections. In South Africa, the first G9 strain was isolated from a sample collected in October 1997. BLASTn analysis revealed the highest homology to the VP7 genes of MW69. The VP4 genes of South African G9 strains were shown to have the highest homology with US1205 (G9P[6]), isolated in the United States during 1996–1997.

A limitation of the South African study was that the selection

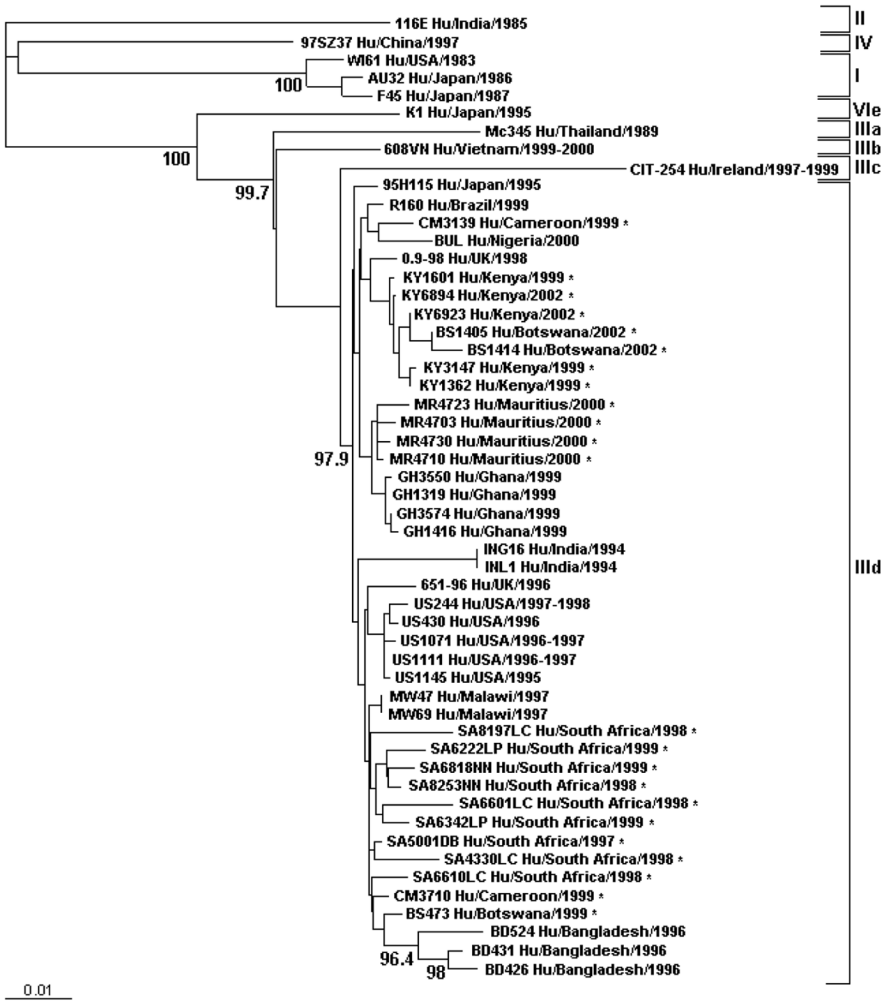

Figure 2. Phylogenetic analysis of the full and partial VP7 nucleotide sequences of African and selected international G9 strains. The length of the abscissa to the connecting node is proportional to the genetic distance between sequences and is indicated by the scale bar. Bootstrap values >90% are indicated at the appropriate nodes. Lineages, as defined by Phan et al [61], are indicated where applicable. *Strains sequenced in this study.

criteria of the stool samples allowed for the identification of only serotype G9 strains with short electropherotypes and SGI specificity. Circulation of G9 strains with long electropherotypes and SGII specificity in South Africa before 1997, therefore, cannot be discounted. In fact, all serotype G9 strains isolated globally during 1983, 1986, 1987, 1994, and 1995 (Figure 1) displayed SGII specificity and long electropherotypes. The SGI, P[6], short electropherotype G9 strains seem to have appeared after 1995, and detection of these strains in southern Africa since 1997 supports this idea.

Serotype G9 rotavirus strains were first isolated from an 18-month-old child with gastroenteritis in Philadelphia, Pennsylvania, in February 1983 [22]. The genogroup characteristics associated with this strain included SGII specificity and a long electropherotype. Subsequently, SGII, long RNA profile G9 strains were isolated in Japan in 1986 and 1987 [24, 54] and in India in 1987 and 1994 [27, 55]. In 1995, a new generation of serotype G9 strains appeared to emerge from various centers, including Bangladesh [56]; Philadelphia, Pennsylvania [57]; and Japan (58, 59) (Figure 1). The reemerging G9 strain from Japan (95H115/95) displayed the characteristic SGII, long electropherotype but grouped phylogenetically with G9 strains isolated in the United States during 1995–1998 that displayed a SGI short electropherotype. Recent studies on serotype G9 strains have shown that these strains are promiscuous, reassorting with SGI, short electropherotype, or SGII long electropherotype strains, while still maintaining an overall VP7 amino acid identity of >90% [58, 60].

The serotype G9 strains detected in Africa were associated with both DS-1–like characteristics (SGI, P[6], and short RNA profile), and Wa-like characteristics (SGII, P[8], and long RNA profile) (Figure 1). The G9 strains isolated in southern Africa tended to display the former genogroup characteristics, whereas strains from Kenya and Mauritius tended to display the latter

genogroup characteristics. Although a G9 strain detected in Botswana in 1999 (BS473) was more closely related to southern African strains, G9 strains isolated in 2002 (BS1405 and BS1414) seemed to be more closely related to those G9 strains with SGII and long electropherotypes found in East and West Africa, despite having SGI specificity and short electropherotypes. These results further support the ideas that (1) serotype G9 strains are promiscuous in nature and reassort frequently and (2) rotavirus strains emerge from a pool of related strains rather than evolve from previous epidemics.

In West Africa, G9 strains displaying either DS-1– or Wa-like characteristics were also identified. Of interest, although the genogroup characteristics did not differentiate strains from Ghana or Kenya, a different picture emerged in Cameroon. Four SGII, long electropherotype G9P[8] strains were collected in the coastal South Western Province, and a single SGI, short electropherotype G9P[4] strain was collected in the drier, mountainous Western Province [42]. These strains also demonstrated different phylogeny, with the SGII, long electropherotype strains clustering with East and West African strains and the SGI, short electropherotype strain clustered with southern African strains.

Analyses of antigenic regions A, B, C, and F revealed point mutation at positions 87 (A→T), 208 (T→I), 220 (A→T), and 242 (T→N) in all African G9 strains (Figure 1). These point mutations were consistent in almost all G9 strains detected after 1994 and seemed to originate from G9 strains detected in India and Japan in 1994 and 1995, respectively. Phylogenetic analyses also clustered all the African G9 strains with international G9 strains isolated after 1994 and in lineage IIId, as defined by Phan et al [61]. In fact, African G9 strains appear to have originated from 2 sources: India in 1994 and Japan in 1995 (Figure 2).

The reaction of 7 South African G9 strains with F45:1 shows that despite the monoclonal antibody being developed against an older G9 strain and amino acid substitutions occurring in the antigenic regions, the antigenic character of the South African strains has not altered sufficiently to allow escape. These results support those of Hoshino et al [62] that the older lineage-1 strains induce broad, cross-reactive, G9-specific antibodies. In addition, F45:1 might prove to be useful in large-scale epidemiological studies in Africa, especially in regions where the cost of reagents and lack of equipment availability prevent the use of molecular techniques for typing.

Despite the maintenance of overall amino acid homology, subtle differences exist in the antigenic composition of the VP7 protein, and the nature and spectrum of anti-VP7 neutralizing antibodies generated by different G9 strains has also been shown to vary [62]. The global emergence of serotype G9 strains has prompted fears that the current vaccine candidates may not protect against these new serotypes. In fact, symptomatic reinfection of vaccinated children with G9 has been shown to be possible [37, 63], and as suggested by Phan et al [61], further classification of G9 strains into lineages and sublineages may aid in the identification of monotypes that escape vaccine-generated immunity.

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